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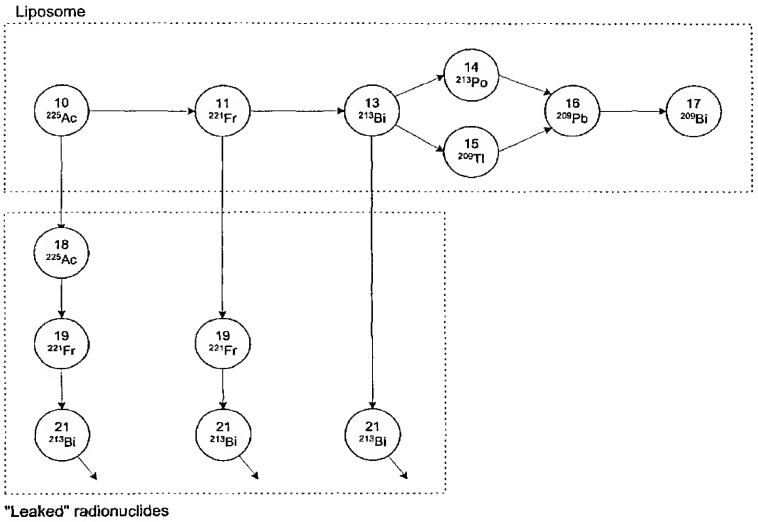
(54) **Title:** LIPOSOMAL ENCAPSULATION OF CHELATED ACTINIUM-225 AND USES THEREOF



(57) **Abstract:** Actinium-225 and other alpha particle-emitting radionuclides hold great promise as potential therapeutic agents for cancer treatment. However, use of these radionuclides is limited by systemic toxicity resulting from release of administered radionuclides and radioactive decay products thereof. If the radionuclides are confined to the target cells, efficacy is increased and toxicity is decreased. However, covalent linking of the radionuclides to targeting molecules does not prevent toxicity resulting from the systemic release of alpha-particle emitting daughter radionuclides. The present invention provides targeted delivery of alpha particle-emitting radionuclides and their alpha-emitting progeny using liposomal encapsulating to prevent the loss of progeny radionuclides from the targeting vehicle and, therefore, the tumor site.

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LIPOSOMAL ENCAPSULATION OF CHELATED ACTINIUM-225 AND USES THEREOF

BACKGROUND OF THE INVENTION

Cross-reference to Related Application

This non-provisional patent application claims benefit of provisional patent application U.S. Serial number 60/212,186, filed June 16, 2000, now abandoned.

Field of the Invention

The present invention relates generally to the field of radiotherapy. More specifically, the present invention relates to liposomal encapsulation of alpha particle-emitting radionuclides. Most specifically, the present invention relates to liposomal encapsulation of chelated actinium-225 and uses thereof.

Description of the Related Art

Optimal treatment with many drugs requires maintenance of a drug level for an extended period of time. For example, optimal anti-cancer treatment with cell cycle-specific antimetabolites requires maintenance of a cytotoxic drug level for a prolonged period of time. The half-life of many drugs after an intravenous (IV), subcutaneous (SC), intraperitoneal (IP), intraarterial (IA), intramuscular (IM), intrathecal (IT), or epidural dose is very short, being in the range of a fraction of an hour to a few hours.

Liposomes are one potential targeting vehicle for drug delivery. Multivesicular liposomes (MVL), first reported by Kim, *et al.* (Biochim, Biophys. Acta, 728:339-348, 1983), are uniquely different from other lipid-based drug delivery systems such as unilamellar (Huang, Biochemistry, 8:334-352, 1969; Kim, *et al.*, Biochim. Biophys. Acta, 646:1-10, 1981) and multilamellar (Bangham, *et al.*, J Mol. Bio., 13:238-252, 1965) liposomes. In contrast to unilamellar liposomes (also known as unilamellar vesicles, or "ULV"), multilamellar and multivesicular liposomes (MVL) contain multiple aqueous chambers per particle. Because of the similarity in acronyms, multivesicular liposomes (MVL) are frequently confused with multilamellar liposomes (MLV). Nevertheless, the two entities are entirely distinct from each other. Whereas multilamellar liposomes (also known as multilamellar vesicles or MLV) contain multiple concentric chambers within each liposome particle, resembling the "layers of

an onion," the multiple aqueous chambers in multivesicular liposomes are non-concentric. The structural differences between unilamellar, multilamellar, and multivesicular liposomes are well known in the art.

The structural and functional characteristics of multivesicular liposomes are not directly predictable from current knowledge of ULV and multilamellar liposomes. The differences are described on page 19 of the book Liposomes as Tools in Basic Research and Industry (Jean R. Philippot and Francis Schuber, eds., CRC press, Boca Raton, Fla., 1995, page 19). Multivesicular liposomes are bounded by an external bilayer membrane shell, but have a very distinctive internal morphology, which may arise as a result of the special method employed in the manufacture. Topologically, multivesicular liposomes (MVL) are defined as liposomes containing multiple non-concentric chambers within each liposome particle, resembling a "foam-like" matrix. The presence of internal membranes distributed as a network throughout multivesicular liposomes may serve to confer increased mechanical strength to the vesicle, while still maintaining a high volume:lipid ratio as compared to multivesicular liposomes. The multivesicular nature of multivesicular liposomes also indicates that, unlike for unilamellar vesicles, a single breach in the external membrane of a multivesicular liposomes will not result in total release of the internal aqueous contents. Thus, both structurally and functionally the multivesicular liposomes are unusual, novel and distinct from all other types of liposomes. As a result, the functional properties

of multivesicular liposomes are not predictable based on the prior art related to conventional liposomes such as unilamellar vesicles and multivesicular liposomes.

The prior art describes a number of techniques for producing unilamellar vesicles and multivesicular liposomes (for example, U.S. Pat. Nos. 4,522,803 to Lenk; 4,310,506 to Baldeschwieler; 4,235,871 to Papahadjopoulos; 4,224,179 to Schneider; 4,078,052 to Papahadjopoulos; 4,394,372 to Taylor; 4,308,166 to Marchetti; 4,485,054 to Mezei; and 4,508,703 to Redziniak). The prior art also describes methods for producing multivesicular liposomes (Kim, et al., Biochim. Biophys. Acta, 728:339-348, 1983). For a comprehensive review of various methods of unilamellar vesicles and multivesicular liposomes preparation, refer to Szoka, et al., Ann. Rev. Biophys. Bioeng.,9:465-508, 1980. In the method of Kim, et al. (Biochim. Biophys. Acta, 728:339-348, 1983), the pharmaceutical utility of multivesicular liposomes encapsulating small therapeutic molecules, such as cytosine arabinoside or cytarabine, is limited. Subsequent studies (Kim, et al., Cancer Treat. Rep., 71:705-711, 1987) showed that the release rate of encapsulated molecules into biological fluids can be modulated by encapsulating in the presence of a hydrochloride.

The prior art is deficient in an effective means for sequestering Ac-225 and its daughter radionuclides at specific targets during radiotherapy. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

Alpha particle-emitting radionuclides hold great promise as potential therapeutic agents for cancer treatment. In treating late-stage breast cancer patients (with measurable liver or bone metastases), long-lived alpha particle emitters are required to reach distant metastases that have developed their own vasculature. One of the most promising such radionuclides, Ac-225 has a 10-day half-life and results in intermediates that yield a total of 4 alpha particles. This radionuclide is shown to be 1000-fold more effective than Bi-213, in animal studies, however, it has also proved to be far more toxic. The increased efficacy and toxicity are a result of the alpha-particle emitting intermediates. When these are confined to the target cells, efficacy is increased, when they distribute throughout the body, toxicity is increased. This is a fundamental difficulty if antibodies are to be used as the targeting vehicle since the bond holding the Ac-225 atom to the antibody will be broken after decay of Ac-225. This will leave the first daughter in the decay chain free to distribute throughout the body where it will decay and subsequently yield additional alpha emissions to normal organs from subsequent daughter decays. In short, of the 4 alphas, only the first one, originating from decay of Ac-225 contributes to the tumor dose, the remainder will distribute throughout normal tissue to increase toxicity. The present invention demonstrates that liposomal encapsulation of Ac-225 reduces loss of radioactive decay intermediates from the targeting vehicle and, therefore, the tumor site.

In one embodiment of the present invention, there is provided a method is provided for preventing the systemic release of radioactive decay intermediates upon administration of an alpha particle-emitting radionuclide to an individual. The radionuclide is incorporated into the aqueous phase of multivesicular liposomes so that the resulting radioactive decay intermediates remain sequestered within the multivesicular liposomes and are not systemically released into the individual. The alpha particle emitting radionuclide may be incorporated into the aqueous phase as a chelation compound. The instant invention is especially useful for the delivery of the alpha particle-emitting radionuclides ^{225}Ac, ^{223}Ra, ^{213}Bi and ^{211}At, and utility of the instant invention for ^{225}Ac delivery has been examined in depth.

In another embodiment, the method of the instant invention is used for the treatment of cancer.

In another embodiment of the instant invention, the liposomes are coated with molecules that preferentially associate with a target cell such as anti-tumor antibodies. As a result, both targeting and target retention of the liposomes are enhanced. One example of such a molecule would be an antibody.

In a further embodiment of the instant invention, retention of radioactive decay intermediates is facilitated by incorporating metal-binding or halogen binding molecules into the aqueous phase of the liposomes.

In yet another embodiment of the present invention, non-tumor specific uptake of radionuclide containing liposomes into reticuloendothelial organs such as the spleen and liver is inhibited by preinjecting an individual with empty liposomes to saturate the nonspecific absorption of liposomes into these organs.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate embodiments of the invention and therefore are not to be considered limiting in their scope.

Figure 1 shows the radioactivity collected in each fraction after Sephadex™ column chromatography at different times after ^{111}In encapsulation.

Figure 2 shows the retention of ^{111}In in liposomes as a function of time after loading.

Figure 3 shows the model of Ac-225 radioactive decay used to determine loss rate of daughter radionuclides from liposome-encapsulated Ac-225 activity.

Figures 4A and **4B** show sample simulations using models of the transfer rate from each sub-compartment within liposomes to the extraliposomal compartment.

Figures 5A, 5B, 5C and **5D** depict simulations obtained using 4 different loss rates.

Figures 6A and **6B** show the relationship between loss rate and the levels of daughter activity at different measurement times after liposome separation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides targeted delivery of alpha particle-emitting radionuclides and their alpha-emitting progeny as it relates, for example, to breast cancer therapy using liposome-encapsulated alpha emitters.

More specifically, the instant invention provides a method of preventing the systemic release of radioactive decay

intermediates upon administration of an alpha particle-emitting radionuclide to an individual by incorporating the radionuclide into the aqueous phase of multivesicular liposomes. The radioactive decay intermediates that remain sequestered within the multivesicular liposomes are not systemically released into the individual. The alpha particle emitting radionuclide may be incorporated into the aqueous phase as a chelation compound. The instant invention is especially useful for the delivery of the alpha particle-emitting radionuclides ^{225}Ac, ^{223}Ra, ^{213}Bi and ^{211}At. The utility of the instant invention has been especially evaluated for ^{225}Ac.

The instant invention is especially directed to the use of the liposomes for the delivery of alpha particle emitting radionuclides for the treatment of cancer.

The present invention also provides a method in which the liposomes are coated with molecules that preferentially associate with a target cell. One example of such a molecule would be an antibody. Anti-tumor antibodies would be especially useful for targeting cancer cells.

The instant invention also includes a method to facilitate retention of radioactive decay intermediates by incorporating metal-binding or halogen binding molecules into the aqueous phase of the liposomes.

The instant invention also provides a method to reduce non-tumor specific uptake of the radionuclide containing liposomes into reticuloendothelial organs such as the spleen and liver by preinjecting empty liposomes into the individual to saturate absorption of liposomes into these organs.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Rationale for liposomal incorporation of alpha emitters

Alpha particle-emitting radionuclides hold great promise as potential therapeutic agents for cancer treatment. To date, only two such radionuclides, astatine-211 (At-211) and bismuth-213 (Bi-213) have been investigated clinically. Both have short half-lives (7 hours and 46 minutes, respectively) and are, therefore, appropriate for situation in which targeting is very rapid. Their use has been limited to leukemia patients, in which the disease is rapidly accessible by intravenous administration (Bi-213), or to patients that have undergone surgical excision of brain cancer, wherein the radionuclide was injected directly into the surgical cavity (At-211). The former study demonstrated the low toxicity of IV-injected alpha emitters, while the latter approach has yielded prolonged survival in poor prognosis

patients. In both studies, the radionuclide was attached to a antibodies against tumor associated antigens. In treating other tumors, such as breast cancer with liver and bone metastases, longer-lived alpha emitters would be required to reach distant metastases which have developed their own vasculature. For such tumors, a more sophisticated targeting approach would be necessary.

Although liposomes have been used in the delivery of chemotherapy and in gene targeting, the use of liposomes in the delivery of radioactivity has not been accepted. This is primarily because high uptake of the liposomes was observed in reticuloendothelial organs such as the liver in the spleen during initial studies performed in the 1980's. Since that time, however, new liposomal systems have been generated with reduced reticuloendothelial uptake. Examples include sterically-stabilized liposomes coated with monosialogangliosides or polyethylene glycol (PEG). The use of such liposomes for the delivery of Ac-225, and other promising alpha emitters such as radium-223 (Ra-223) is particularly compelling because they may retain daughter radionuclides with the aqueous phase and thereby reduce systemic toxicity. Since range of alpha particle (50-100 microns) is sufficient to penetrate beyond the liposomal membranes (70nm), tumor irradiation will be enhanced.

EXAMPLE 2

Analysis of indium-111 leakage from liposomes

To optimize the liposomal formulation and evaluate potential leakage of radionuclides from liposome constructs, indium-111 was used in place of Ac-225. Indium-111 is easily detected by gamma counting and served to develop and test the methodology for subsequent encapsulation of Ac-225.

Both Indium-111 and actinium-225 form tri-chloride complexes.

The method described by Hwang *et al.* was used with minor modifications. Liposomes were isolated by Sephadex™ chromatography and loaded with ^{111}In by incubation for 1 h at room temperature with a loading solution consisting of 6-10 µl 6.9 mM oxine in oxine sulfate in deionized water with 200 µl 1.8% NaCl/20mM sodium acetate, pH 5.5. To this acetate buffer, an equal volume of $^{111}InCl_3$ in 3 mM HCl was added to make the final loading solution. Loading was terminated by passage through an AGIX ion-exchange column. The fractions corresponding to liposomes were pooled and stored at 4°C and 37°C for evaluation of indium leakage.

At different times after ion-exchange chromatography, aliquots of the pooled liposomal fractions were drawn, chromatographed by size exclusion (Sephadex™ column) separation and counted for radioactivity in a gamma counter. The

counts in liposomal fractions were expressed as a fraction of the radioactivity aliquoted from the original pooled sample. In selected samples, prior incubation with DTPA was included to ensure that retention of ^{111}In in liposomal fractions was not the result of leakage and equilibration of ^{111}In between extra- and intra-liposomal ^{111}In in the pooled sample.

EXAMPLE 3

Results from the indium-111 experiments

Following ion-exchange chromatography, approximately 20% of the radioactivity remains on the ion-exchange resin, and close to 80% in the liposomal fractions, yielding an encapsulation efficiency of approximately 80%. The radioactivity collected in each fraction after Sephadex™ column chromatography at different times after ^{111}In encapsulation is depicted in **Figure 1**. The results show a generally time-invariant profile following size-exclusion separation.

Figure 2 depicts the retention of ^{111}In in liposomes as a function of time after loading. The fraction of ^{111}In retained within liposomes appears to remain constant at approximately 80% over a prolonged time-period, indicating that retention of Ac-225 within liposomes is possible.

EXAMPLE 4

Analysis of actinium-225 leakage from liposomes

The next step in this work was to carry out these same experiments using Ac-225. In this case, gamma counting provided information regarding the presence of daughter radionuclides within the liposomes. Liposomes were incubated for 1 hr with Ac-225. To determine loss rate of daughter radionuclides from liposome-encapsulated Ac-225 activity, Ac-225 containing liposomes were separated from free Ac-225 by elution through a Sephadex™ column. The fractions corresponding to the liposomes (fractions 4-6, based on In-111 studies) were pooled and counted on a gamma counter using windows appropriate for detection of the Fr-221 and Bi-213 photopeaks (218 and 440 keV, respectively). A 1-minute counting interval was selected and counting was performed overnight. Fr-221 counts were decay corrected to the start of the 1-min. counting period. At the end of separation and 10 to 20 h later (if possible), the column were also counted in a dose calibrator.

The results were analyzed using the model of Ac-225 radioactive decay shown in **Figure 3**. Sub-compartments 10-17 correspond to decays that occur within liposomes. Loss of daughter (or parent) radionuclide is depicted by a "leak" rate from the liposome compartments to corresponding sub-compartments in the "leaked" radionuclides compartment. The transfer rate from each sub-compartment within liposomes to the extraliposomal compartment reflects a rate of loss from liposomes.

This rate may be described in terms of a loss or clearance half-life, the amount of time required for Ω of a particular radionuclide to diffuse out of the liposome. Transfer rates within a compartment correspond to physical decay, whereas those that cross between the two compartments correspond to transfer of radionuclide from one to the other compartments. Since astatine-217, which has a 32 msec half-life is not resolved by this model, it is lumped with Fr-221.

A sample simulation using this model is depicted in **Figures 4A** and **4B**. **Figure 4A** shows the results of a simulation in which complete retention of all radionuclides was assumed. Results are expressed relative to the initial activity of Ac-225 encapsulated by the liposomes. The first solid line shows the decay of Ac-225 while the other solid curves show the rise in daughter radioactivity within liposomes as equilibrium between the parent and daughters is reached. Note that because of its short, 5 min half-life, Fr-221 achieved equilibrium much more rapidly than Bi-213, which has a 45.6 min half-life. The two dotted lines, corresponding to Fr-221 or Bi-213 activity not within liposomes, are just visible at zero throughout the simulation duration.

Figure 4B shows a simulation assuming loss of Fr-221 at a rate of 0.046 min^{-1}, which is equivalent to a loss half-life of 15 min. All other loss rates were set to zero. This means that Bi-213

generated within liposomes was assumed to remain there. Complete retention of Ac-225 was also assumed in this simulation.

As shown by the rise in both liposome-associated and free daughters (solid and dashed lines, respectively), equilibration and loss of daughters was allowed to occur during the 1-hr incubation period. After separation, and during counting of liposomal fractions, the distinction between free and liposome-encapsulated radioactivity was no longer possible. Therefore, the loss rate is turned off so that the solid curves correspond to total daughter radioactivity associated with the liposomal fractions (including daughters that were leaking out); the dashed curves reflect the physical decay of daughter radionuclides remaining in the column or in the liposome-free fractions. Although the latter could be assayed for radioactivity of Fr-221 and Bi-213, the method for assessing daughter loss rates was based upon counting the liposomal fractions.

EXAMPLE 5

Analysis using different loss rates

Figures 5A-5D depict simulations obtained using 4 different loss rates. The curves associated with counting of liposomal fractions are shown without the corresponding curves for free daughters collected on the column or in the liposome-free fractions. All other conditions are as described above. The CPM

values are normalized by dividing by CPM expected after 10 h (i.e., at equilibrium).

In **Figure 5A**, (loss half-life = 15 min) the curves corresponding to a simulation that did not include loss of Fr are shown for comparison with the simulation that included loss. The level of Fr-221 and Bi-213 activity immediately after separation was sensitive to the loss half-life of Fr-221. This is clearly evident for Fr-221 since the loss rate impacted the equilibrium level (plateau) that was reached. It was less evident for Bi-213 since it does not reach equilibrium in 1 h. Once the separation had occurred (i.e., after 60 minutes), the distinction between free and liposome-associated daughter activity was lost over time as the daughters reached equilibrium with the parent. Correspondingly, as the measurement of daughter activity was delayed relative to the time of separation, the ability to distinguish the different loss rates is also reduced.

EXAMPLE 6

Relationship between loss rate and daughter activity

The relationship between loss rate and the levels of daughter activity at different measurement times after liposome separation is shown more directly in **Figure 6**. **Figure 6A** shows the normalized count rate ratio, as presented in **Figures 5A-5D**, of each daughter divided by the corresponding count rate ratio

assuming no loss of Fr-221 and then subtracted from one to yield curves that approach zero over time. Results are plotted against different loss half-lives and for measurement times of 15, 30 and 60 minutes after separation. If counting is started immediately after separation and carried out overnight, these data are available.

Figure 6B is a different representation of the data used to generate **Figure 6A**. Loss rate sensitivity is plotted against the time post-separation at which the liposomal fractions are counted. Curves are provided for three different loss half-lives: 30, 60 and 180 min.

EXAMPLE 7

Determine the loss rate that yields a negligible absorbed dose to kidneys from Bi-213

To determine the loss rate that yields a negligible absorbed dose to kidneys from Bi-213, a 5 day biological half-life of the Ac-225 construct is assumed and a 25 d simulation is plotted. Subsequently, the Bi-213 released for different Fr-221 release rates is integrated and used to perform rough estimate of dosimetry. 25 d = 36000 min = simulation time 5 d half–life = 9.627e-5

EXAMPLE 8

Possible enhancements of liposomal delivery of alpha emitters

Three further enhancements in the use of liposomes to deliver alpha emitters to tumors are also specifically contemplated within the context of the methods of the present invention. One is to incorporate metal-binding or halogen-binding molecules within the aqueous phase of the liposomes to bind and enhance retention of daughter radionuclides. A second enhancement is the preinjection of large, empty liposomes to saturate the reticuloendothelial organs to reduce non-tumor specific spleen and liver uptake of radionuclides. The third and final approach is to coat the liposomes with anti-tumor antibodies to increase tumor localization and retention of the liposomes

The following references were cited herein:

Hwang, K. J., et al. "Encapsulation, with High Efficiency, of Radioactive Metal Ions in Liposomes." Biochim Biophys Acta 716.1 (1982): 101-9.

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. It will be apparent to those skilled in the art that various modifications and variations can be made in practicing the present invention without departing from the spirit or scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A method of preventing the systemic release of radioactive decay intermediates upon administration of an alpha particle-emitting radionuclide to an individual in need of such treatment, comprising the steps of:

incorporating said radionuclide into the aqueous phase of liposomes; and,

administering said liposomes to said individual, wherein radioactive decay intermediates remain sequestered within said liposomes.

2. The method of claim 1, wherein said alpha particle emitting radionuclide is incorporated into the aqueous phase as a chelation compound.

3. The method of claim 1, wherein said alpha-particle-emitting radionuclide is selected from the group consisting of ^{225}Ac, ^{223}Ra, ^{213}Bi and ^{211}At.

4. The method of claim 3, wherein said alpha particle-emitting radionuclide is ^{225}Ac.

5. The method of claim 1, wherein said individual is being treated for cancer.

6. The method of claim 1, wherein said liposomes are coated with molecules which preferentially associate with a specific target cell to augment the targeting and target retention of said liposomes.

7. The method of claim 6, wherein said molecules are antibodies.

8. The method of claim 7, wherein said antibodies are anti-tumor antibodies.

9. The method of claim 1, wherein additional molecules are incorporated into the aqueous phase of said liposomes to facilitate retention of radioactive decay intermediates, wherein said molecules are selected from the group consisting of metal-binding molecules and halogen binding molecules.

10. The method of claim 1, wherein said individual is preinjected with empty liposomes to saturate the

reticuloendothelial organs to reduce non-tumor specific spleen and liver uptake of said radionuclide.

11. The method of claim 1, wherein such liposomes are either unilamellar or multilamellar.

12. The method of claim 1, wherein auxilliary molecules are included in the liposome formulation in order to prevent uptake by the reticuloendothelian organs.

13. The method of claim 12, whereing the auxilliary molecules are polyethyleneglycol-linked lipids (PEG-lipids).

14. The method of claim 6, wherein the targeting molecules are attached to auxilliary molecules.

15. The method of claim 1, wherein additional molecules are incorporated into the formulation in order to faciliatate membrane fusion with target cells or to facilitate endocytosis by target cells.

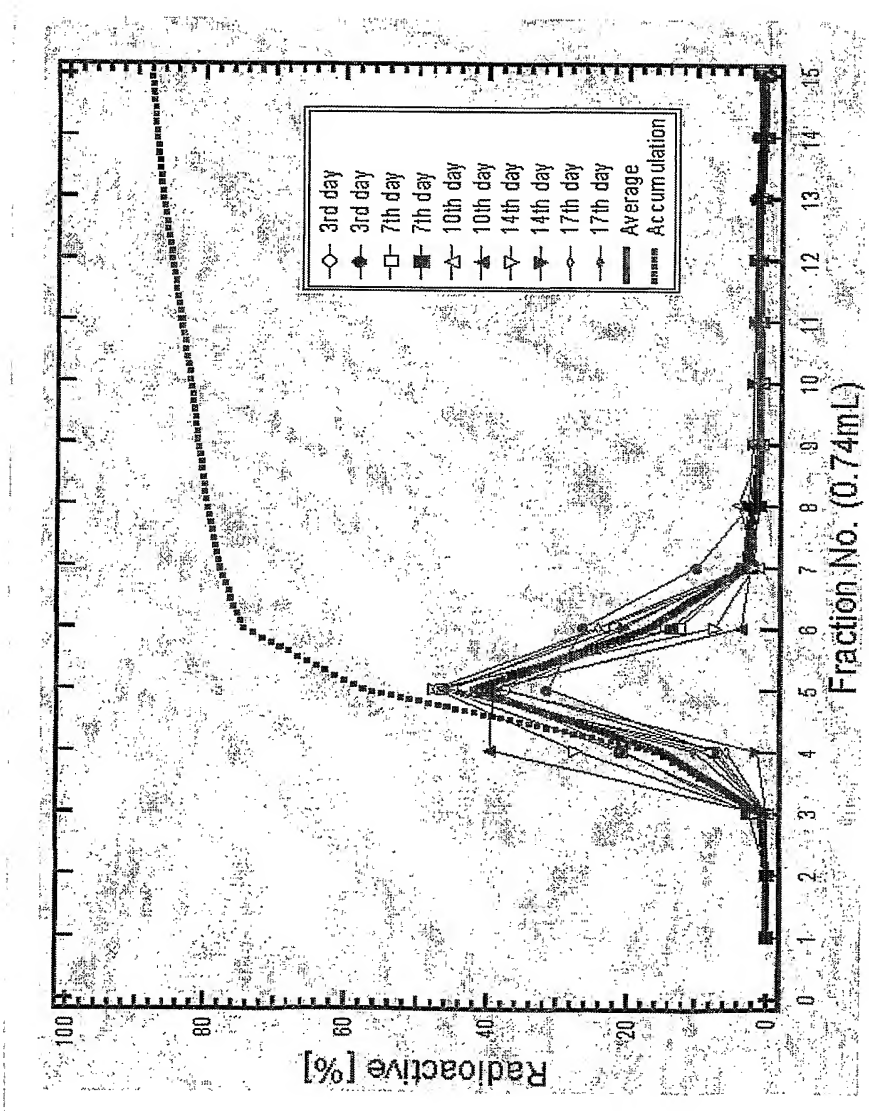
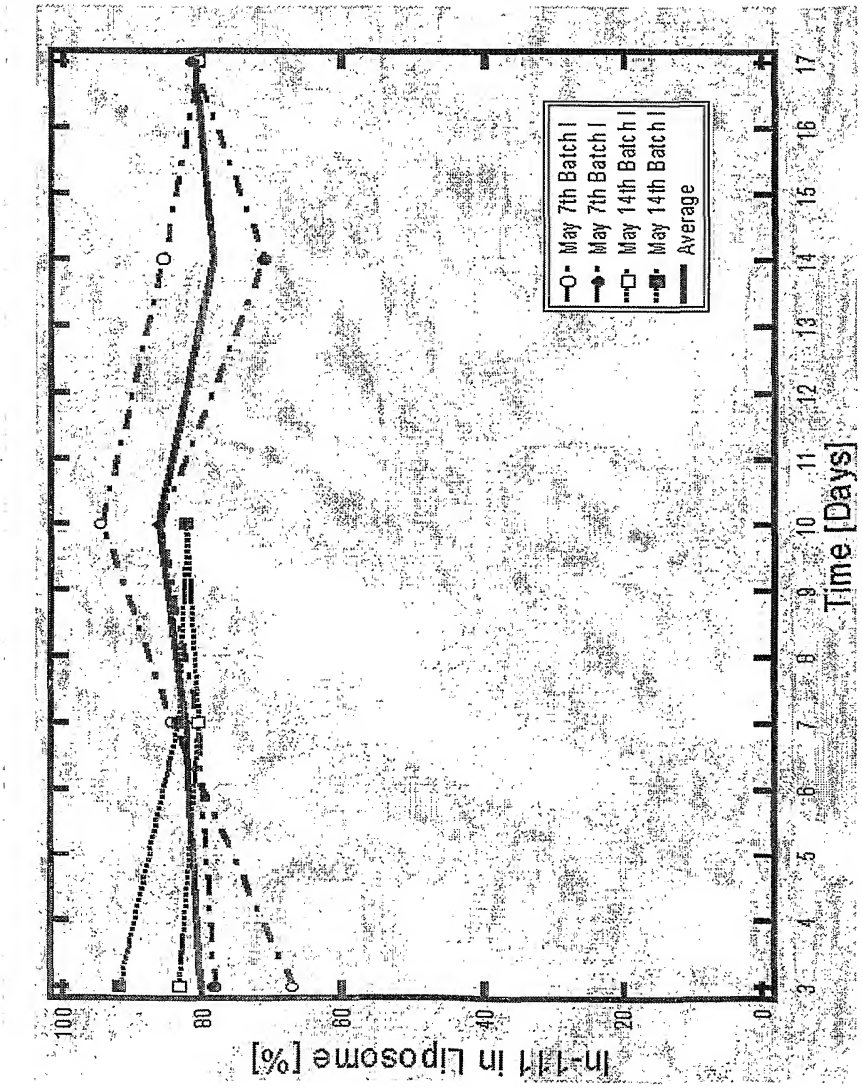

Radioactive [%]
Fraction No. (0.74mL)
3rd day
3rd day
7th day
7th day
10th day
10th day
14th day
14th day
17th day
17th day
Average
Accumulation

Fig. 1


In-111 in Liposome [%]
Time [Days]
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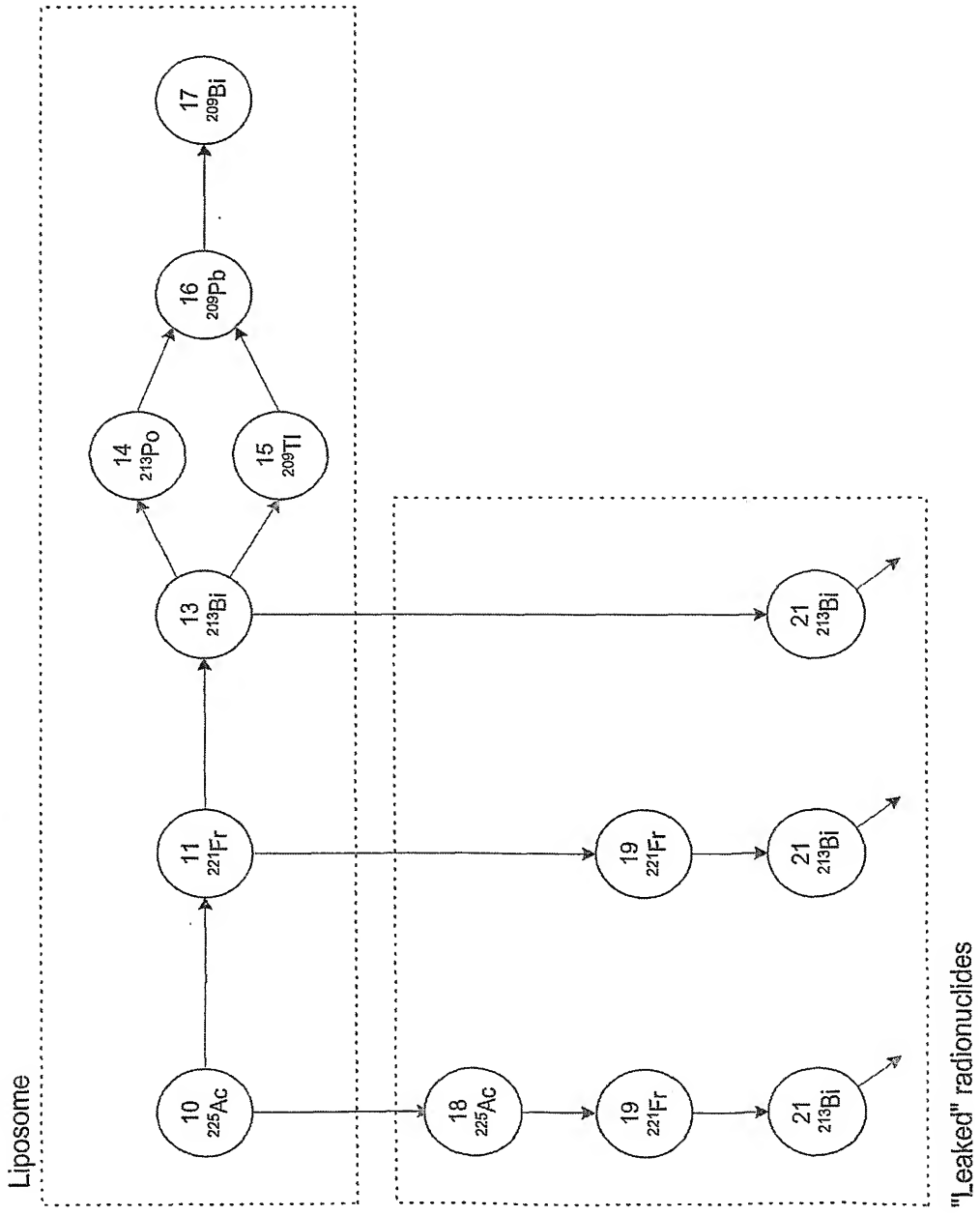
May 7th Batch I
May 14th Batch I
May 14th Batch I
Average

Fig. 2


Liposome
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11 221Fr
13 213Bi
14 213Po
15 209Tl
16 209Pb
17 209Bi
18 225Ac
19 221Fr
19 221Fr
21 213Bi
21 213Bi
21 213Bi
"Leaked" radionuclides

Fig. 3

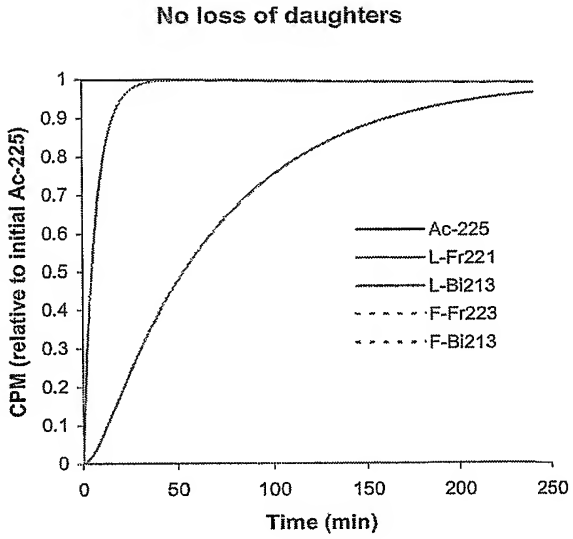
No loss of daughters
CPM (relative to initial Ac-225)
Time (min)
Ac-225
L-Fr221
L-Bi213
F-Fr223
F-Bi213

Fig. 4A

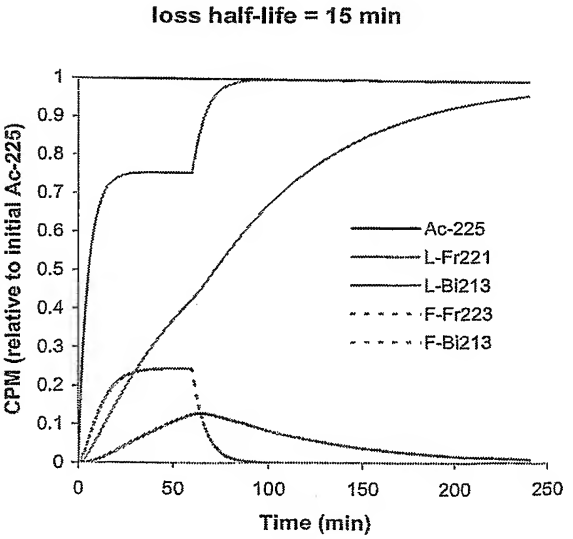
loss half-life = 15 min
CPM (relative to initial Ac-225)
Time (min)
Ac-225
L-Fr221
L-Bi213
F-Fr223
F-Bi213

Fig. 4B

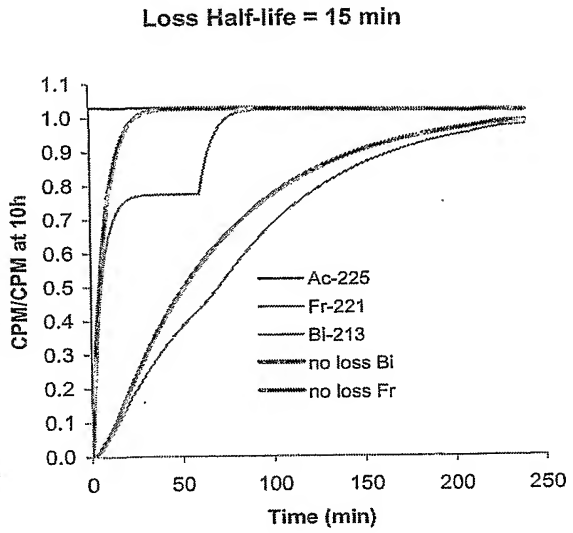
Loss Half-life = 15 min
CPM/CPM at 10h
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
50
100
150
200
250
Time (min)
Ac-225
Fr-221
Bi-213
no loss Bi
no loss Fr

Fig. 5A

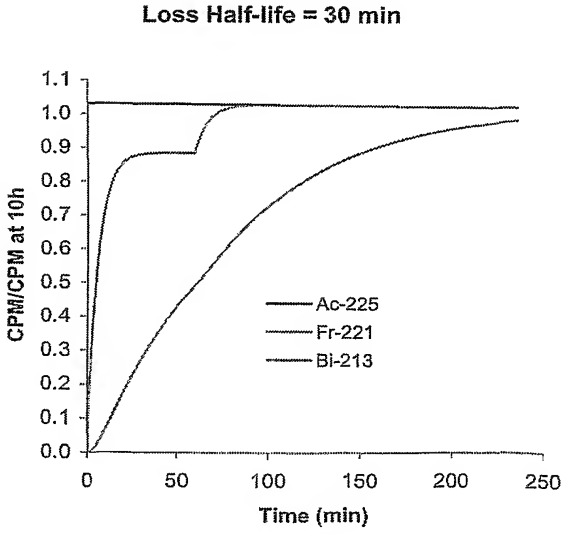
Loss Half-life = 30 min
CPM/CPM at 10h
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
50
100
150
200
250
Time (min)
Ac-225
Fr-221
Bi-213

Fig. 5B

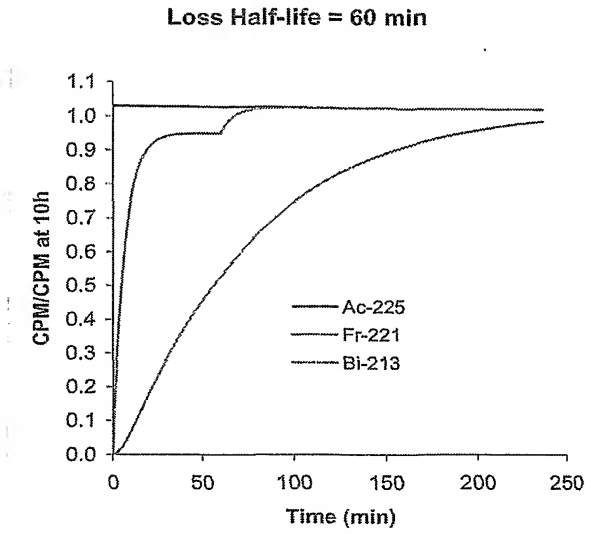
Loss Half-life = 60 min
CPM/CPM at 10h
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
50
100
150
200
250
Time (min)
Ac-225
Fr-221
Bi-213

Fig. 5C

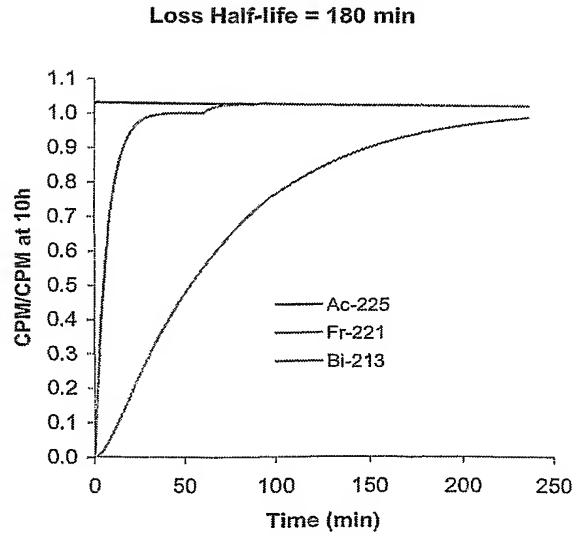
Loss Half-life = 180 min
CPM/CPM at 10h
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
50
100
150
200
250
Time (min)
Ac-225
Fr-221
Bi-213

Fig. 5D

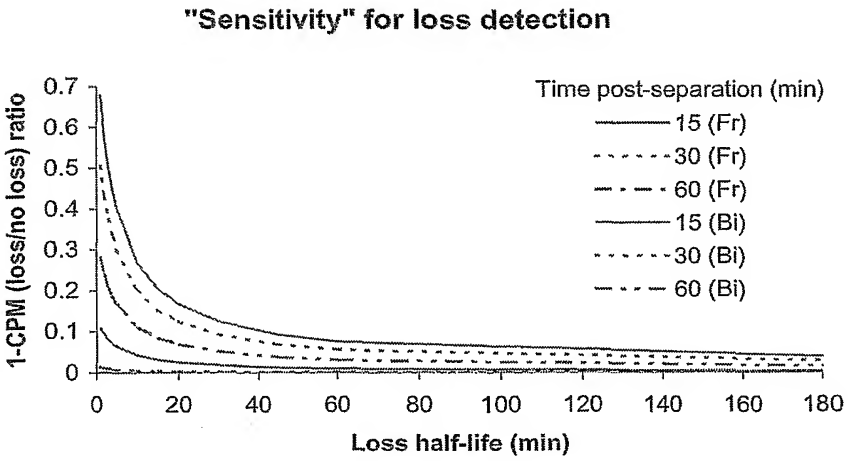
"Sensitivity" for loss detection
1-CPM (loss/no loss) ratio
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
120
140
160
180
Loss half-life (min)
Time post-separation (min)
15 (Fr)
30 (Fr)
60 (Fr)
15 (Bi)
30 (Bi)
60 (Bi)

Fig. 6A

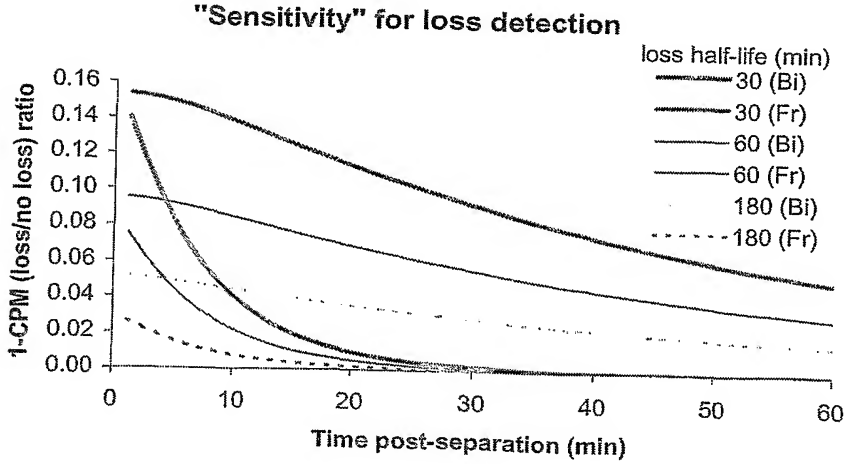
"Sensitivity" for loss detection
1-CPM (loss/no loss) ratio
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
60
Time post-separation (min)
loss half-life (min)
30 (Bi)
30 (Fr)
60 (Bi)
60 (Fr)
180 (Bi)
180 (Fr)

Fig. 6B

INTERNATIONAL SEARCH REPORT

Ir ional application No.
PCT/US01/19133

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 51/00, 51/12, 9/127, 103/00
US CL :424/1.21, 9.32, 450

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.21, 9.32, 450

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, STN ONLINE, CAPlus, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 4,665,897 A (LEMELSON) 19 May 1987, abstract, col 14, lines 50-57, col 16, lines 22-29.	1, 5-7, 11 --- 2, 9-10, 15
X	US 5,328,678 A (FUJII et al) 12 July 1994, abstract, col 5-8.	1-2, 5, 11
Y	US 5,853,752 A (UNGER et al) 29 December 1998, col. 21, lines 22-40, col. 25, lines 15-55, col. 35, lines 25-31, col. 40, lines 59-67, col. 41, lines 1-25.	1-15
Y	US 6,060,315 A (HOLCOMB et al) 09 May 2000, abstract, col. 17, lines 20-56.	1-15
Y	US 5,534,241 A (TORCHILIN et al) 09 July 1996, abstract, col. 5 lines 5-65.	1-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 SEPTEMBER 2001	Date of mailing of the international search report 15 NOV 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SHAHNAM SHARAREH Telephone No. (703) - 306-5400

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

Ir ional application No.
PCT/US01/19138

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,624,846 A (GOLDENBERG) 25 November 1986, abstract, col. 18, lines 44-67.	1-15
X, E	WO 2001060417 A2 (LARSEN et al) 23 August 2001, abstract.	1-15
Y	US 4,394,372 A (TAYLOR) 19 July 1983, abstract, col 5-6.	1-15